

1/18

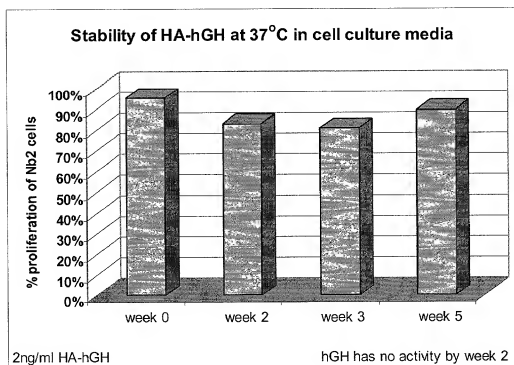
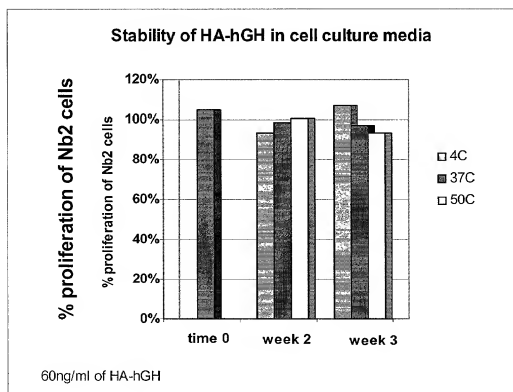


Figure 1

**Figure 2**

3/18

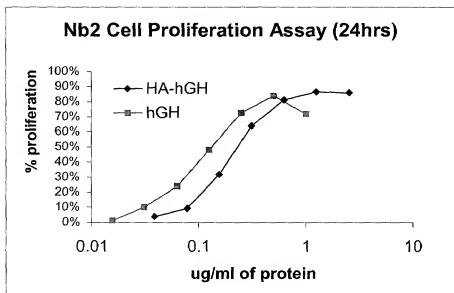


Figure 3A

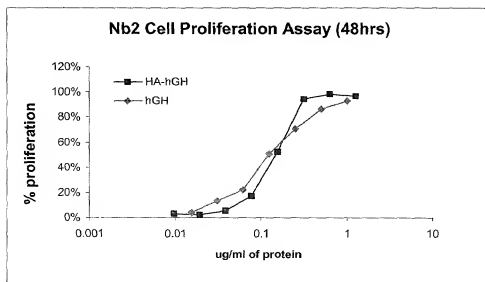


Figure 3B

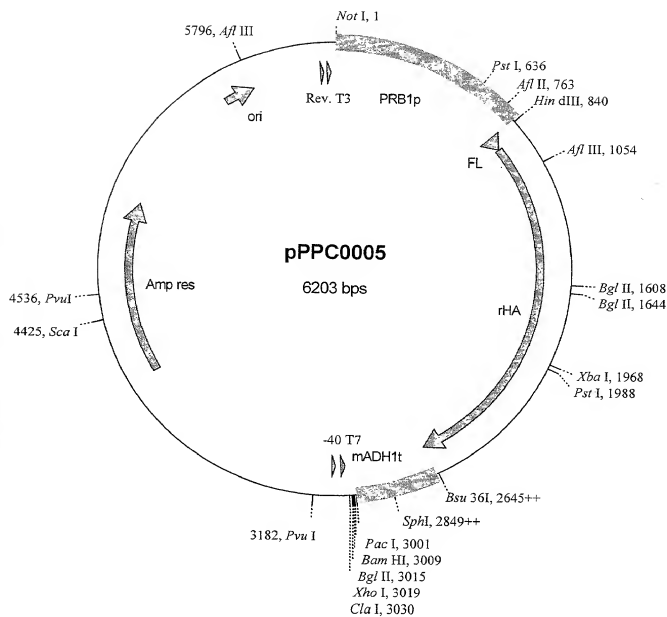


Figure 4

5/18

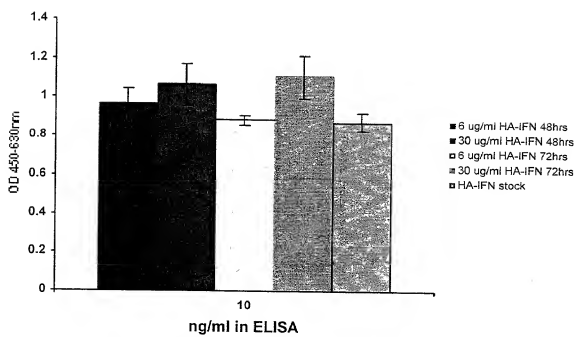


Figure 5

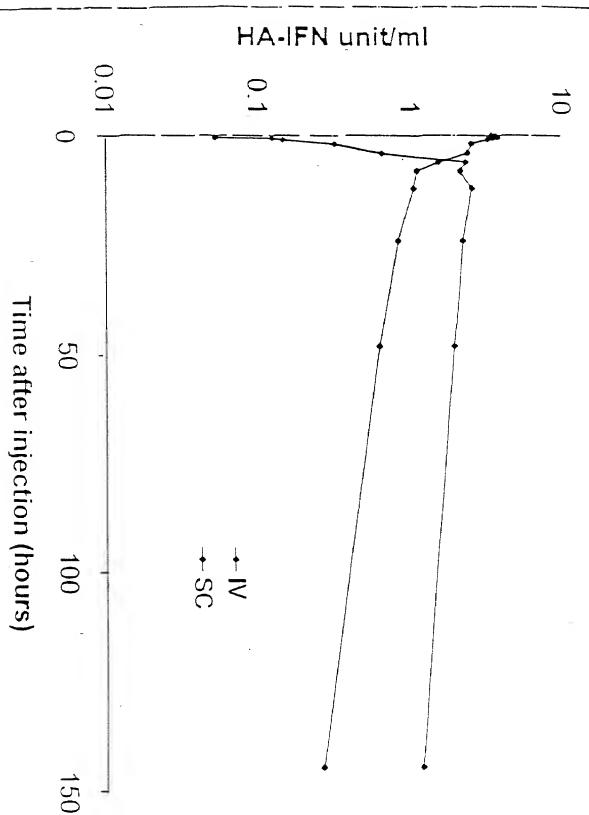
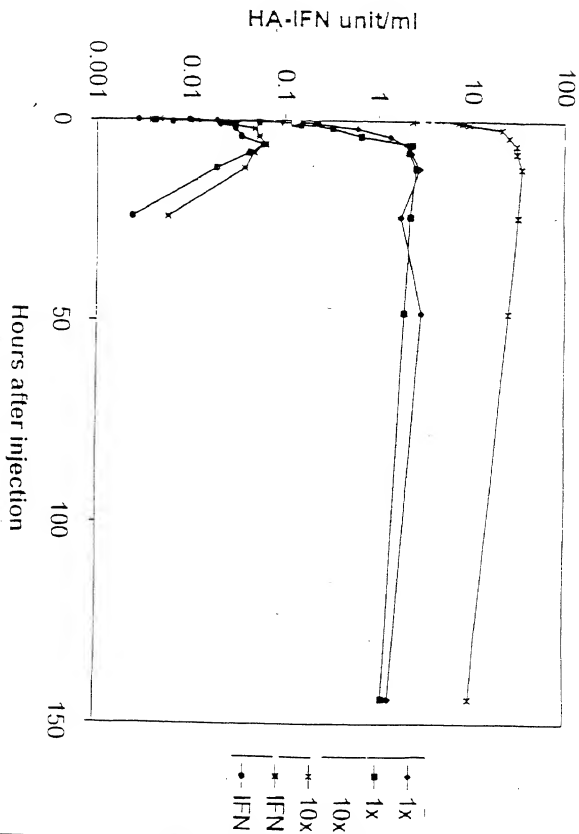


Figure 6

[illegible]

Figure 7



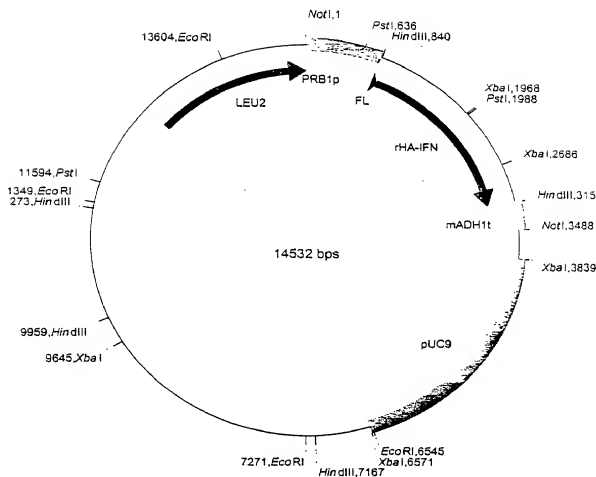


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises

PRB1 promoter, from *S. cerevisiae*.

Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF α -1 leader.

HA-IFN α coding sequence with a double stop codon (TAATAA)

ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind III*/*Bam*HI fragment generally used.

Figure 8

9/18

Localisation of 'Loops' based on the HA Crystal Structure
which could be used for Mutation/Insertion

1	DAHKSEVAHR	FKDLGEENFK	ALVLIAPAFQY	LQQCPFEDHV	KLVNEVTEFA
	HHHHH	HHH	HHH	HHHHHHHHHH	HHHHH
	I		II		III
51	KTCVADESAE	NCDKSLHTLF	GDKLCTVATL	RETYGEMADC	CAKOEPPERNE
	HHHHH	HHHHH	HHHHH	HHHH	H
101	CFLQHKDDNP	NLPRLVRPEV	DVMCTAFHDN	BETFLKKYLY	EIARRHPYFY
	HHHH	H	HHHHHHHH	HHHHHHHHH	HHHHH
	IV				
151	APELLFFAKR	YKAAFTECCO	AADKAACLLP	KLDELRLDEGK	ASSAQORLKC
	HHHHHHHHHH	HHHHHHHHH	HHHHH	HHHHHHHHHH	HHHHHHHHHH
	V				
201	ASLQKFGERA	FKAWAVARLS	QRFPKAEFAE	VSKLVTDLTK	VHTECCCHGDL
	HHHHH	HH	HHHHHHHHHH	HH	HHH
	VI		VII		
251	LECADDRADL	AKYICENODS	ISSKLKECCF	KPLLEKSHCI	AEVENDEMPA
	HHHHHHHHHH	HHHHH	HHHHH	HHHHHHH	H
301	DLPSLAADFV	ESKDVCKNYA	EAKDVFLGMF	LYEYARRHPD	YSVVLRLRLA
	HHHH	HHHHHH	HHHHHHH	HHHHHH	HHHHHHHH
	VIII				
351	KTYETTLEKC	CAAADPHECY	AKVFDEFKPL	VEEPQNLIKQ	NCELFEQLGE
	HHHHHHHHHH	HH	H	HHHHH	HHHHHHHHH
	IX				
401	YKFQNALLVR	YTKKVPQVST	PTLVEVSRNL	GKVGSKCCKH	PEAKRMPCAE
	HHHHHHHHHH	HHHH	H	HHHHHHHHHH	HHH
	X		XI		
451	DYLSVVLNQL	CVLHEKTPVS	DRVTKCCTES	LVNRRPPCFSA	LEVDETYVPK
	HHHHHHHHHH	HHHHH	HHHHHHHHH	HHHHHHHH	
501	EFNAETFTFH	ADICTLSEKE	RQIKKQATALV	ELVKHKPKAT	KEQLKAVMDD
		HHH	HHH	HHHHHMEHHH	HHH
	XII				
551	FAAFVEKCKC	ADDKETCFAE	EGKKLVAASQ	AALGL	
	HHHHHHHH	HHHH	HHHHHHHHHH	HH	
	Loop		Loop		
	I	Val54-Asn61	VII	Glu280-His288	
	II	Thr76-Asp89	VIII	Ala362-Glu368	
	III	Ala92-Glu100	IX	Lys439-Pro447	
	IV	Gln170-Ala176	X	Val462-Lys475	
	V	His247-Glu252	XI	Thr478-Pro486	
	VI	Glu266-Glu277	XII	Lys560-Thr566	

Figure 9

Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

```

151  APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH          HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

IV

```

151  APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH          HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

$(X)_n$



IV

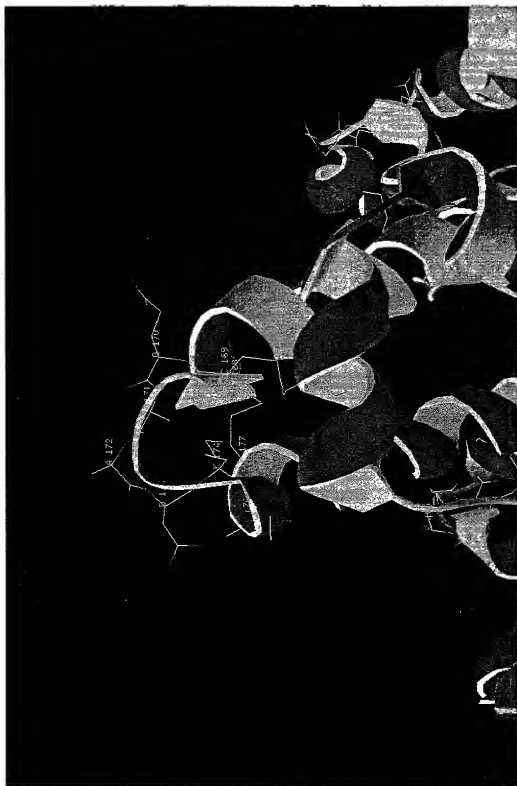
```

151  APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHHH          HHHHH HHHHHHHHHHH HHHHHHHHHH
  
```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10





Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176

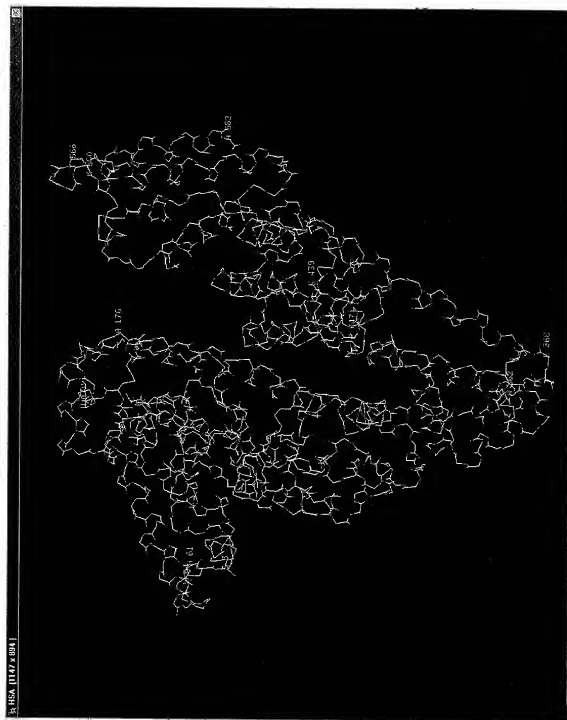


Figure 13: Tertiary Structure of HA

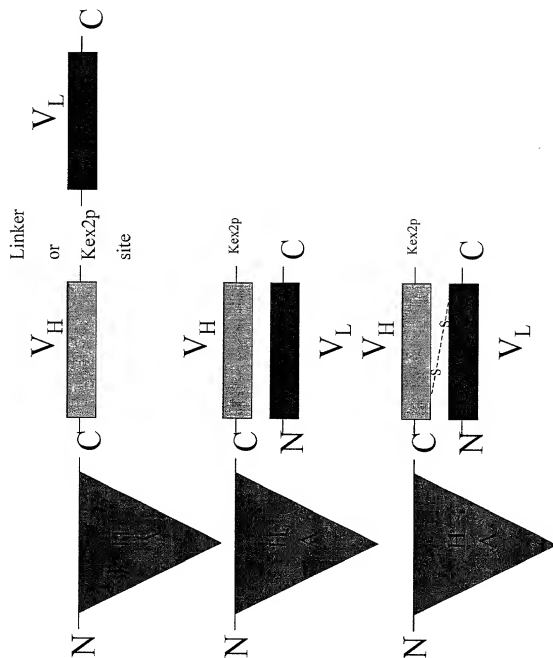


Figure 14: Schematic Diagram of Possible ScFv Fusions
 (Example is of a C-terminal fusion to HA)

15/18

1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60
 1 D A H K S E V A H R F K D L G E N F K 20

61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
 101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
 121 D V M C T A F H D N E E T F L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
 141 E I A R R H P Y F Y A P E L L F F A K R 160

Figure 15A

16/18

481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A S L Q K K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAT TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A K Y I C E N Q D S I S S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

17/18

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGG CAT CCT GAT 1020
 321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y S V V L L L R L A K T Y E T T L E K C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C A A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
 461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

18/18

1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L V N R R P C F S A L E V D E T Y V P K 500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E F N A E T P T F H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R Q I K K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K E Q L K A V M D D F A A F V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A D D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCC TTA GGC TTA TTA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A A L G L * 585

Figure 15D